A STUDY OF MUSTARD VESICATION*

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Vesication in man is a common phenomenon, induced by a variety of mechanisms, among them mechanical, thermal, infective and chemical. The latter attracted great interest following the introduction of "poisonous" gases to the weapons of warfare, and has been responsible for a considerable effort to elucidate the mechanisms by which the skin is injured. This has been particularly true of the mustards, the greatest interest centering on sulphur mustard.† Only in isolated cases have "acceptable" instances of vesicles due to mustard been noted in animals, all under unusual circumstances. Various attempts have been made to explain this seeming species tissue difference, but none has been completely satisfactory.

METHOD

Suckling rats, which are hairless until the 7th day of life, were used except in a few instances in which hairless mice, possessing skin with a texture resembling that of man, were used. The skin of the back of rabbits and rats following epilation by Victamul‡ was studied and six suckling pigs up to 10 days of age were also studied, using the clipped skin of the back and the skin of the ears. (The ears are sparsely hirsute.) Most exposures were carried out by the vapor cup method. To accommodate the variable size of animals, glass cups with a mouth diameter varying from 0.4 to 1.5 cm. were made. The base of the cup was slightly expanded so that a small disc of filter paper could be retained, the filter paper being saturated with sulphur mustard a short time before use. Initially, exposures were made by pressing the cup to the skin for periods of from 30 seconds to 15 minutes. In a few instances, daily 2 minute exposures were made for 4 days. Later vapor cup exposures were made only after the skin was moistened with saline.

Exposures were also made by direct skin contact of mustard on neonatal suckling rats and hairless mice. Small discs of filter paper were affixed to strips of Scotch tape and saturated with mustard, which was then placed on the back of the animal and left in place for periods varying from 30 seconds to 6 minutes. Later in the period of study, the skin was moistened with saline before applying the mustard by this method.

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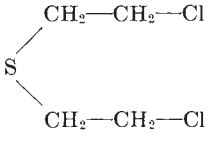
† 

‡ Victor Chemical Company—A detergent.

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RESULTS

Initially, nothing resembling a vesicle was produced by a vapor cup exposure in suckling rats. Erythema was detectable within a few hours and reached a maximum by 24 to 36 hours. The redness, however, never extended significantly beyond the area of the burn. By 72 hours, the erythema had subsided, the area becoming somewhat pigmented and contracted with a slight central depression. The late lesion (7 days) presented a slightly brownish, hyaline appearance with scaliness and alopecia which was permanent.

Microscopically, these lesions were characterized by hyperplasia of the epidermis, atrophy of hair follicles and inflammatory changes, primarily in the dermis. The granular layer was particularly notable by its thickness and was associated with hyperkeratosis. Polyploidy of nuclei in the germinativum was frequently noted. The epidermal thickening was usually more marked near the margins, (Fig. 1).

During this initial phase of the study, one 5 day old rat developed a lesion, noted at 44 hours, distinctly different from any previously seen, (Fig. 2). The central zone of this lesion, corresponding exactly to the size of the vapor cup used (4 mm.), had a greyish-white discoloration and was sharply demarcated by an 0.25 cm. margin of erythema; viz., a lesion simulating a human vesicle although there was no elevation by fluid. Inadvertently, the section of this lesion was destroyed during histological preparation; but a litter mate of this rat had been sacrificed at 25 hours following exposure, sections of which were available for histological examination. It was found that changes of an entirely different character from those noted previously were present and consisted of vacuolate areas within the germinativum and focal areas of necrosis of basal cells, associated, in some instances, with local separation of the epidermis from the dermis (Figs. 3 and 4). This experience could not be repeated in four successive litters of rats varying in age from 2 to 7 days. The possibility arose that there was some relation between the moistness of the skin and the production of a vesicle-like lesion. To test this, five rats from another litter were exposed by the vapor cup method after moistening the skin with saline and all developed vesicle-like lesions within 24–36 hours identical in appearance to the one noted above.

Following this observation, the skin was routinely moistened with saline, the excess being wiped away, before vapor cup exposures. In subsequent studies on suckling rats from 12 different litters, it proved to be an exceptional circumstance when vesicle-like lesions did not develop. It is noteworthy that when failure did occur, the whole litter showed a uniform pattern. An exact figure for the incidence of "vesication" by this method is not possible since the animals were sacrificed at varying intervals, often before demarcation could be expected. But a general appraisal would indicate at least 75% success. It was found by trial that "vesicles" could be produced by 5 minute vapor cup exposure, but were most consistently produced by 8 minute exposures.

The following is a general description of the evolution of the mustard burn lesion in the suckling rat, produced by the technic described above. Erythema is readily apparent within 5 hours following exposure, covering an area some-

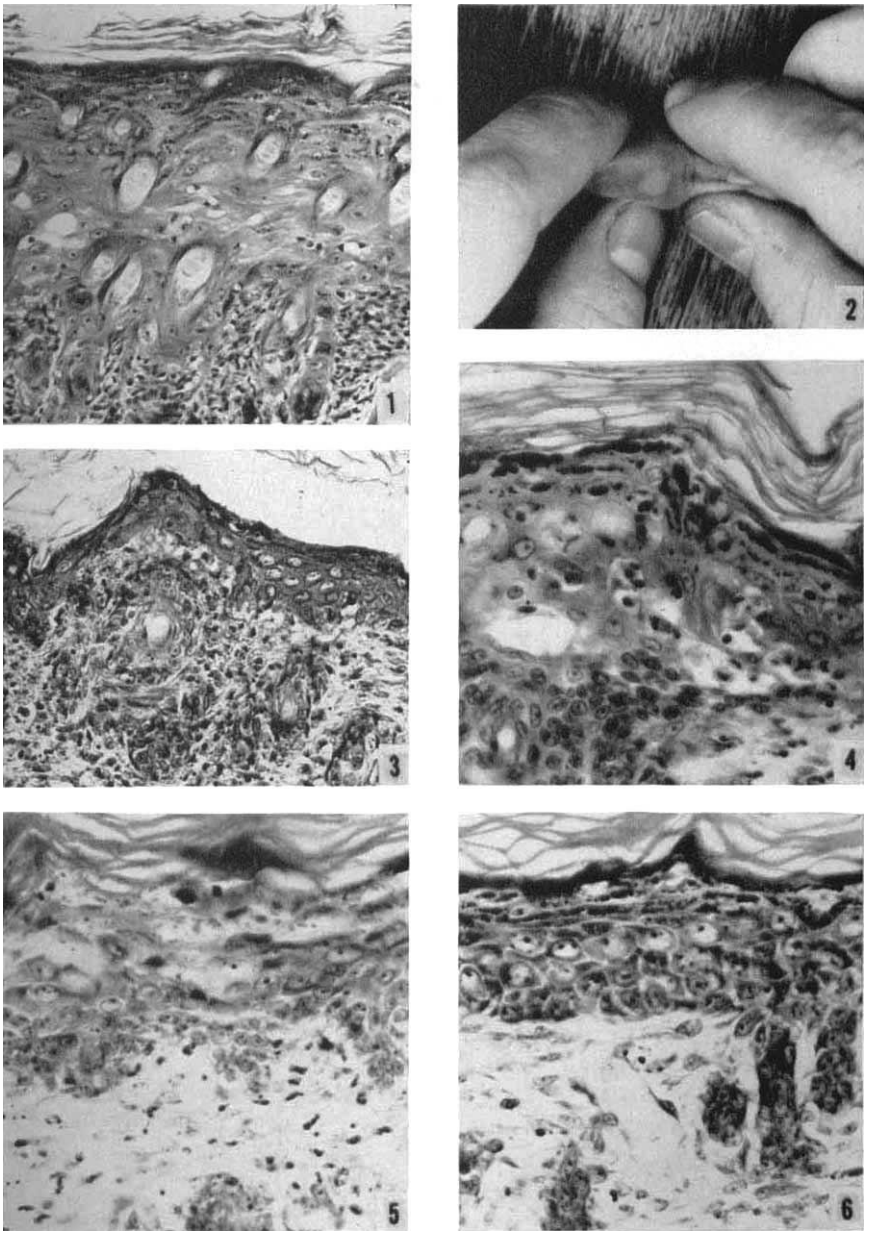


FIG. 1. Hyperplasia of the epidermis 5 days following a 10 minute exposure of a 4 day old rat to mustard vapor. Note the prominence of the granular layer. Much of the S. corneum has split away in preparation. This appearance is well established by 36–48 hours. Hematoxylin and Eosin ×200.

FIG. 2. The appearance at 48 hours of the vesicle-like lesion produced by vapor cup exposure to mustard.

FIG. 3. Selective damage of the basal cell layer and hair follicles in a 6 day old rat exposed 10 minutes to mustard 24 hours previously. Hematoxylin and Eosin ×225.

FIG. 4. Vacuolation in the Germinativum of a 4 day old rat, 26 hours after an 8 minute exposure of the moistened skin to mustard. Hematoxylin and Eosin ×400.

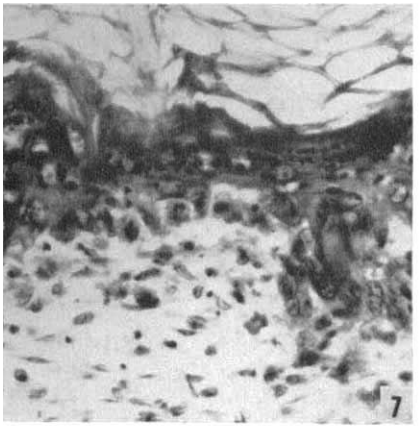
FIG. 5. Extreme loss of cytoplasmic basophilia and necrobiosis, particularly marked in the lower strata and basal cell layer of the Germinativum and in hair follicles. Eight minute exposure of the moistened skin to mustard vapor, 23 hours previous. May-Gruenwald Giemsa ×400.

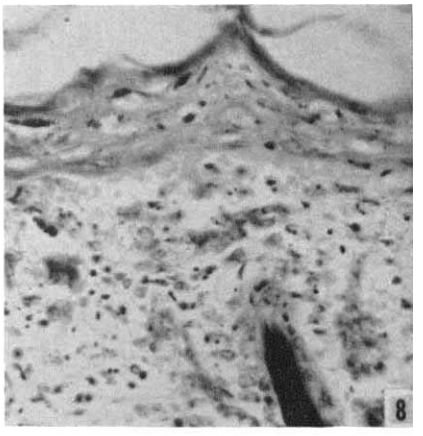
FIG. 6. A normal area of the same section illustrated in Fig. 5 for comparison. May-Gruenwald Giemsa ×400.

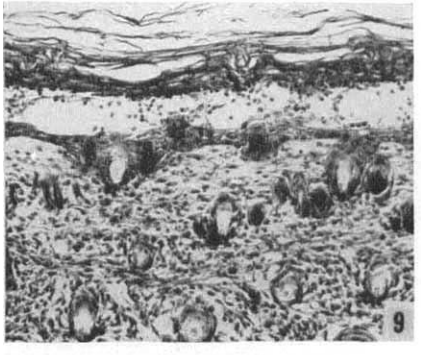
what larger than that actually exposed. There is then little change until approximately 18–20 hours at which time beginning demarcation of the exposed area can be observed, initially in the form of patches of bluish-grey discoloration. These coalesce in the next 24–48 hours producing a bluish-grey area, precisely the size of the vapor cup, sharply demarcated by the surrounding border of erythema (Fig. 2). This sharply demarcated zone soon becomes less translucent and more greyish-white in color. The lesion then becomes brownish in color, the marginal demarcation less distinct and there is a subsidence of the erythematous border. By 96 hours, the lesion is almost plaque-like and is beginning to show desquamation. A brownish, thickened area remains for several days and hair growth fails to appear.

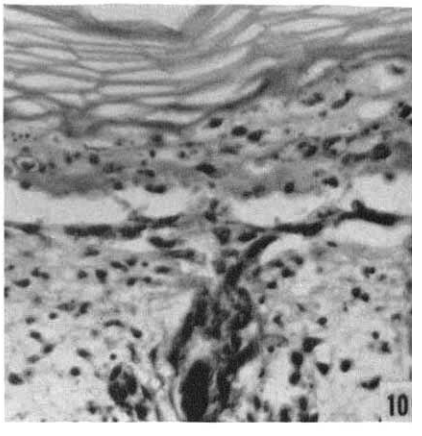
Microscopically, the earliest detectable alterations are congestion in the corium at the dermo-epidermal junction and edema of the corium, a change easily recognized by 5 hours. In the next few hours there is an apparent loss of chromatin of nuclei in the germinativum, particularly in the basal cells, a change which is also notable in portions of the hair follicles. Giemsa stained preparations show not only a loss of nuclear chromatin, but also of cytoplasmic chromatin material, (Figs. 5 and 6). Basophilic debris (karyorrhetic fragments) is to be found in the upper dermis and in hair follicles among the epithelial cells. These changes are quite apparent by 7 hours, at which time, however, very little inflammatory reaction in the form of polymorphonuclear leukocytic infiltration is in evidence. Changes within the epidermis in succeeding hours vary in time of development from animal to animal. Necrobiotic changes become manifest in the basal cells, (Fig. 7) and in Giemsa preparations karyorrhetic fragments may be noted. This necrotizing process may be very well developed by 16 hours and evidence of separation from the dermis at this level is often apparent. In the other strata of the epidermis there is an apparent loss of basophilism and the nuclei have a "naked" appearance, the nuclear membrane seeming to enclose a vacuole in which there are only nucleoli (Fig. 8). Vacuolation of the epidermis is usually observed by this time although it varies in degree. Such spaces seem accountable by disintegration of cells, not by a coalescence of cytoplasmic vacuolation in neighboring cells, almost always extending from the basal cell layer and frequently reaching well into the upper germinativum. There does not appear to be any relation between the inflammatory component of the lesion, which is variable in intensity, and the tendency to separation of the epidermis from the dermis. Acantholysis does not appear to be a significant phenomenon. Everything considered, the epidermis, separate of the basal cells, seems relatively spared.

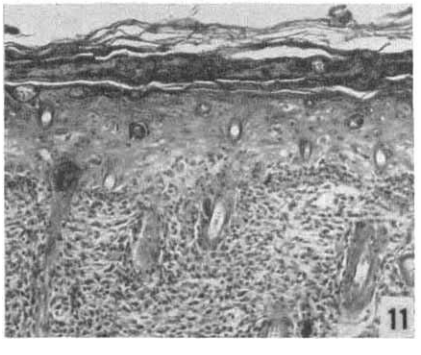
With complete disintegration of the basal cells, which is usually manifest by 20–25 hours, there follows an abrupt separation of the epidermis from the dermis a process, it is to be emphasized, entirely intra-epidermal. In effect, a vesicle is formed, the roof of which consists of the partially necrobiotic epidermis and the floor of denuded dermis. By 26 hours, this process may be advanced and accounts for the gross appearance at this time. The vesicle space contains exudative material of variable character from a simple fibrino-protein nature to one frankly purulent (Fig. 9).











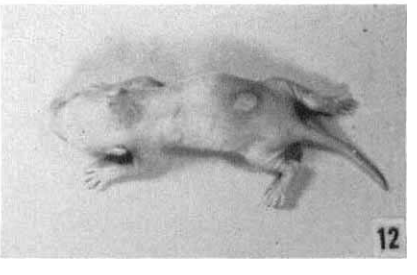


FIG. 7. Selective necrosis of cells in the basal and lower layers of the Germinativum, 16 hours after an 8 minute mustard vapor exposure of the moistened skin in a 3 day old rat. Note the relatively slight acute inflammatory reaction. Hematoxylin and Eosin ×400.

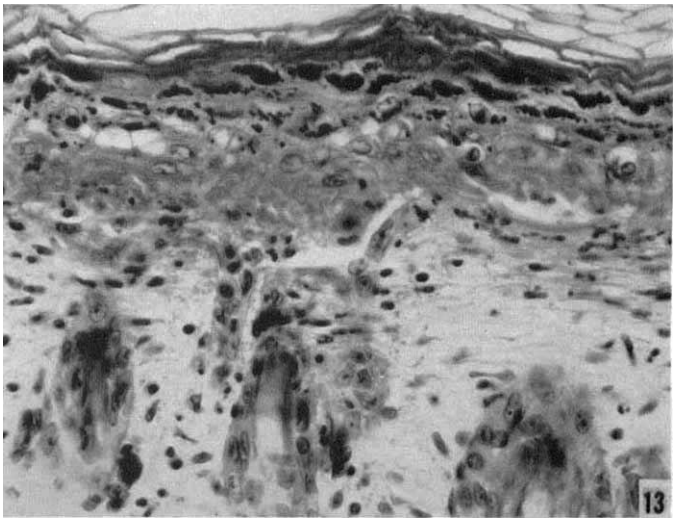
FIG. 8. Complete disintegration of the basal cell layer and marked loss of nuclear and cytoplasmic chromatin in other epithelial cells. Note the scattered nuclear debris at all levels of the section. Three day old rat, 24 hours after an 8 minute exposure of the moistened skin to mustard vapor. May-Gruenwald Giemsa ×400.

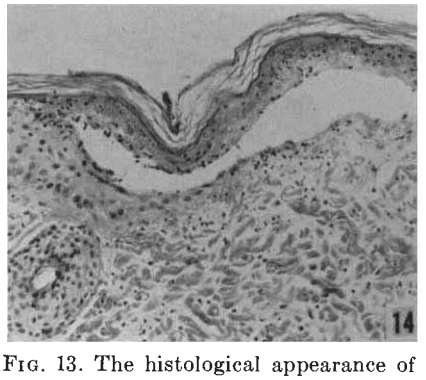
FIG. 9. Separation and moderate elevation of the epidermal plate 48 hours after exposure of the moistened skin of a 4 day old rat to mustard vapor. Note that considerable regeneration of epidermis has occurred. Hematoxylin and Eosin ×110.

FIG. 10. Regeneration of epidermis arising from proliferation of cells in the neck of hair follicles. Three day old rat 43 hours following exposure of the moistened skin to mustard vapor for 8 minutes. May-Gruenwald Giemsa ×400.

FIG. 11. Skin of an 8 day old rat, 96 hours after exposure of the moistened skin to mustard vapor for 8 minutes. Note the condensed layer of separated epidermis overlying the hyperplastic regenerated epidermis. Hematoxylin and Eosin ×110.

FIG. 12. A vesicle-like lesion in a 6 day old rat produced by direct contact of mustard (disc method) 48 hours previously.





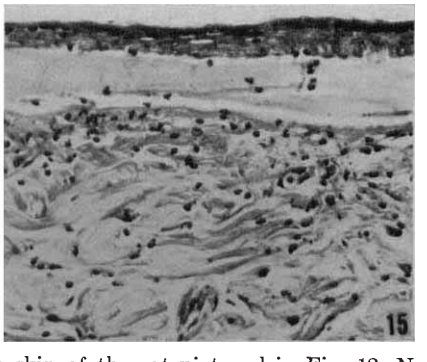


FIG. 13. The histological appearance of the skin of the rat pictured in Fig. 12. Note the very striking selectivity of damage in the basal and lower layers of the Germinativum. Also note the early evidence of regenerating cells from the neck of the hair follicles. Hematoxylin and Eosin ×400.

FIG. 14. A "vesicle" on the ear of 12 day old pig produced by exposure of the moistened skin to mustard vapor for 5 minutes, 46 hours previously. Note the minimal inflammatory reaction and the hyperplasia of the marginal epithelium. Hematoxylin and Eosin ×110.

FIG. 15. A "vesicle" in an adult rat epilated by Victamul 89. Direct contact of mustard (disc method) to the moistened skin 25 hours previously. Hematoxylin and Eosin ×110.

Regeneration of epithelium over the denuded dermis is soon apparent, being marked as early as 44 hours and having its origin in the necks of hair follicles (Fig. 10). It is noteworthy that the cells of the upper portion of the hair follicles seem more resistant to mustard damage than those of the bulb. It is this regeneration of epithelium over the base of the denuded dermis that accounts for the change in appearance of the gross vesicle from a translucent bluish-grey to an opaque greyish-white. In the subsequent course of the lesion the separated epi-

dermis undergoes coagulative changes and there is considerable hyperplasia of the underlying regenerating epidermis (Fig. 11), well marked by 4 days. Later stages were not studied in detail.

The results of using the disc method of producing lesions on suckling rats were much more variable, similar lesions to those above being produced in approximately 50% of some 40 suckling rats from six litters (Figs. 12 and 13). Two experiments were performed on 6 and 8 suckling rats respectively, from which it was found that prior moistening of the skin enhanced the tendency to vesicate by this method as well.

Three suckling pigs, 4 days of age, were exposed to mustard vapor by the cup method after moistening the skin of the back 3 min., 5 min. and 10 min. respectively. At 48 hours the 5 minute "burn" appeared erythematous without clearly defined central demarcation although it was similar microscopically to the typical "vesicle" in the suckling rat. Further studies on three 10 day old suckling pigs were made, 8 min. exposures on the moistened back being compared with 5 min. exposures on the moistened ear. Features pointed out for the neonatal rat lesions could be found in lesions of both sites at 46 hour (Fig. 14).

The results of studies on 13 hairless mice, 10 of which received vapor cup and 3 disc method exposures were, in the most part, of little value. For a given exposure, the skin of the back was much more severely damaged than in any of the other species studied.

Three epilated rabbits and three epilated rats showed quite interesting results. The rabbits received cup exposures of 3, 8 and 12 minutes, the rats an 8 minute cup and a 6 minute disc exposure, all to moistened skin. The sites of intermediate duration in the rabbits and all the sites in the rats showed vesicle-like lesions similar to those in neonatal rats. This was true histologically as well as grossly although the damage to both epidermis and dermis was greater in the rabbit. It was particularly interesting to find a vesicle produced by the disc method on one rat in which the inflammatory component was negligible (Fig. 15).

DISCUSSION

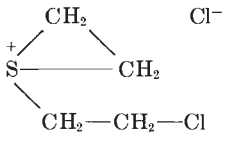
The pathogenesis of the vesicle following mustard injury has been set forth on the basis of the findings in man, utilizing lesions of both accidental and experimental origin. The limitations imposed by the apparent inability to duplicate the phenomenon of mustard vesication in the laboratory or domesticated mammal has resulted, however, in an incomplete understanding of the mechanism by which the epidermis is separated from the dermis. For purposes of orientation it is profitable to compare the experimental mustard lesion described in this report with the lesion in man (5, 7, 10). Although physiological alterations can be demonstrated within 20 minutes of exposure in man and erythema can be detected by one hour, the earliest microscopic changes are not apparent until 3–6 hours. At this time, nuclear changes in the malpighian layer of the epidermis are apparent, consisting of swelling and an apparent decrease in basophilia, correlated with a loss of Feulgen reactive material (7), most striking in the cells of the basal layer. Vacuolate areas develop in the epidermis, particularly in basal

locations and are commonly believed to derive from vacuolar degeneration of the cytoplasm. In keeping with this concept the further changes are designated as a process of liquefaction necrosis (or hydropic degeneration). These vacuolate areas coalesce by 10–12 hours to form microvesicles, manifested in the gross lesion by a "pigskin"-like appearance.

The earliest foci of "liquefaction necrosis" are said to be at the tips of papillae although this is not clearly demonstrated by illustrations. The degenerating basal cells at this time have pyknotic nuclei; karyorrhexis is stated to be seldom noted in the epidermis, although it is a very conspicuous finding in hair follicles. The "dissolution" of cells in the basal layer progresses, there is coalescence of micro-vesicles and by 16–24 hours or more, a macro-vesicle results. Maximum vesicle size is noted in 48–72 hours. The blister fluid is stated to be composed of detritis and edema or tissue fluid. Although the hair follicles show considerable degenerative changes, it is notable that sweat ducts show relatively little change, and sweat glands and sebaceous glands no changes from exposures of vesicant intensity.

The most striking effect noted in both the human lesion and the experimental lesion described in this report is the relative selectivity of damage. In presumably non-lethal concentration, there is a stimulatory effect on the epidermis leading to striking hyperplasia. It is of interest that Fell was unsuccessful in an attempt to produce skin tumors with mustard (1). In lethal concentration (i.e., vesicant quantities) there is necrosis almost exclusively of the basal cells. Both observations add confirmation to the belief that the "young" or reproducing cell is the susceptible cell; i.e., mustard exhibits a radiomimetic effect (3). It is of considerable interest that the kind of lesion produced is in large part dependent on the moisture of the skin (8). There is considerable evidence, through analogy with the nitrogen mustards, that mustard is activated in water, the presumed active form being the ethylene sulfonium ion* (3). Although there is a very limited solubility of sulphur mustard in water it is many fold that leading to cell death. One might well question the significance of the moistness of the skin surface since the cells themselves lie in an aqueous medium. There are no data in this study that give an answer to this question, but conceivably it is related to the lipid solubility of mustard. The noting of typical vesicles accidentally acquired near the teat of lactating bitches (6) constitutes one of the rare examples of vesication produced in animal species other than man. One would expect such skin to be particularly apt to be moist, and it is noteworthy that specialized glands, intermediate between sweat glands and lactiferous glands, the glands of Montgomery, are present in this location.

It is evident from the results of this study that the separation of the epidermis from the dermis in the experimental animal is a result of destruction of basal

* 

cells. In studies on corneal electrical resistance after HN_2 (nitrogen mustard), there is no change until actual separation of the epithelium occurs, an observation suggesting that cell death and not alteration of the cell membrane is responsible for the loss of resistance (3, 4). The loss of cohesion then is to be attributed to a generalized effect on the cell, and not to a specific alteration.

Recognizing that the fundamental lesion in vesicant injury is primarily necrosis of the basal cell layer of the epidermis, how can the subsequent changes be accounted for, particularly those responsible for the chief difference between man and laboratory animals, (viz. a gross blister)? There are two main categories of information suggesting that gross blister fluid can be attributed to the action of sweat glands. Sparing of sweat glands and particularly of the sweat duct in human vesication has been commented on. In its exit through the epidermis the sweat duct is a spiral canal enclosed only by surrounding epidermal cells and it is not difficult to see how this can be interrupted. There are obvious similarities, e.g., of the clusters of intraepidermal vesicles found in situations of hyperhidrosis and the early "pig-skin" lesion of mustard injury (9).

The second category of observations concerns the lack of significant elevation by fluid of the vesicles in experimental animals. Prevalent views explain this as related to the thinness of the epidermis of common laboratory animals and an anchoring of the epidermis by hair follicles (2). However, even in situations where the skin has been made hairless and the epidermis thickened, significant elevation (viz. a fluid containing vesicle) has not resulted. If effusion is the explanation, it is not clear why, in the so called "doughnut" lesion,* there is no central elevation. This cannot always be explained as due to coagulation necrosis as is demonstrated by histologic illustrations of "doughnut" lesions. It is not known if the sweat gland is significantly altered in these severe lesions, but with deep damage to the dermis one would presume this to be the case.

To best account for the manner by which vesicles are produced by mustard injury in man, and to harmonize this with the lesion produced in laboratory animals, two fundamental alterations seem the most significant. The first of these is the direct effect on the mustard sensitive cell, leading to necrobiosis. The second is the indirect effect of disrupting the continuity of the sudoriporous excretory system without affecting its secretory capacity. In most mammals, the lack of true sudoriporous glands (9) would seem to account for the fact that actual blister formation does not occur although in every other respect the lesion is identical to that in man. Such other considerations as the thickness of the epidermis have, at best, indirect roles. The thinness of the epidermis in animals, however, may well account for the general experience by previous investigators of marked dermal injury as contrasted to man. Indeed, the experience with the hairless mouse, as noted in this report, was such as to suggest this.

I wish to express my appreciation to Cpl. Jerome Mandel for his assistance in this study and to Mr. John Cuculis for the photography.

* Such lesions result from severe exposures and are characterized by a ring-shaped blister surrounding a non-vesicated central zone.

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